

Amendments to the Claims:

Please cancel claim 21 without prejudice or disclaimer.

Please amend claims 1, 5, 6, 22, 27, 28, 32, 33, 37, 45 and 46 as follows:

1. (Three Times Amended) A method for the amplification of RNA, in a sample, comprising:

a) obtaining a starting solution by adding to a container [comprising] the sample, a buffer, a first primer, a second primer, a plurality of nucleotide triphosphates, a sufficient amount of an enzyme system having reverse transcriptase activity and a heat stable enzyme system having DNA polymerase activity, [and closing the container,] wherein said sufficient amount is an amount which, after the heat treatment of step b) below, will retain sufficient reverse transcriptase activity to permit performance of step [c)] d) hereafter;

b) heating the solution obtained in a) to a temperature sufficient to permit denaturation, said temperature not to exceed 75° C., and maintaining said temperature for a sufficient time to provide denaturation of said RNA without inactivating the enzyme system having reverse transcriptase activity;

c) permitting the first primer to hybridize with said RNA in said solution;

d) bringing the solution obtained in [b)] c) to a predetermined temperature from 55° to 75°C and maintaining said temperature for sufficient time whereby a first cDNA strand is synthesized and a RNA-cDNA heteroduplex is formed;

[d)] e) heating the solution obtained in [c)] d) to a predetermined temperature whereby said RNA-cDNA heteroduplex is denatured to form an RNA single strand and a first cDNA single strand;

[e)] f) bringing the solution obtained in [d)] e) to a predetermined temperature and maintaining said temperature for a sufficient time whereby the second primer hybridizes with the first cDNA single strand;

[f)] g) bringing the solution obtained in [e)] f) to a predetermined temperature and maintaining said temperature for a sufficient time whereby a second cDNA strand is synthesized to form a double-stranded cDNA; and

[g)] h) denaturing the double-stranded cDNA to form cDNA single strands and subjecting the cDNA single strands to a sufficient number of amplification cycles to obtain a desired amount of amplified product;

wherein after step a), all steps are performed without subsequent addition of any ingredients.

5. (Twice Amended) The method as claimed in claim 1, wherein in step [c)] d) said predetermined temperature is between [50] 55° and 65° C.

6. (Twice Amended) The method as claimed in claim 5, wherein in step [c)] d) said predetermined temperature is between [50] 55° C. and 60° C.

22. (Three Times Amended) A method for the amplification of RNA in a sample, comprising:

a) obtaining a starting solution by placing, in a container, the sample, a buffer, a first primer, a second primer, a plurality of nucleoside triphosphates, and an enzyme system having reverse transcriptase activity and DNA polymerase activity;

b) heat treating said solution at a temperature sufficient to permit denaturation of secondary structures that may be present in said RNA but not above 75° C, for a time sufficient to permit denaturation of secondary structures without completely inactivating the reverse transcriptase and DNA polymerase activities of said enzyme system;

c) permitting the first primer to hybridize with the RNA in said solution, followed by synthesis, at a temperature from 55° to 75° C, of a first cDNA strand, thus forming an RNA-cDNA heteroduplex;

d) heat treating the solution containing said RNA-cDNA heteroduplex at a temperature at which said heteroduplex is denatured to form an RNA single strand and a first cDNA single strand without completely inactivating the DNA polymerase activity of said enzyme system;

e) permitting the second primer to hybridize with the first cDNA single strand, followed by synthesis of a second cDNA strand to form a double-stranded cDNA; and

f) denaturing the double-stranded cDNA to form cDNA single strands and subjecting the cDNA single strands to a sufficient number of amplification cycles to obtain a desired amount of amplified product;

wherein after step a), all steps are performed without subsequent addition of any ingredients.

27. (Amended) The method of claim 22, wherein the RNA to be amplified is human immunodeficiency virus RNA.

28. (Amended) The method of claim 26, wherein said temperature at which said heteroduplex is denatured is above 90° C.

32. (Twice Amended) The method of claim 22, wherein said first cDNA strand is synthesized and said RNA-cDNA heteroduplex is formed at a temperature from 55° C to about 65° C.

33. (Amended) The method of claim 22, wherein the second primer is permitted to hybridize with the first cDNA single strand at a temperature from about 50° C to about 80° C.

37. (Amended) A method for the amplification of RNA in a sample, comprising:
a) obtaining a starting solution by placing, in a container, the sample, a buffer, a first primer, a second primer, a plurality of nucleoside triphosphates, and an enzyme system having reverse transcriptase activity and DNA polymerase activity;

b) heat treating said solution at a temperature of from 60° to 75° C, for a time sufficient to permit denaturation of secondary structures without completely inactivating the reverse transcriptase and DNA polymerase activities of said enzyme system;

c) permitting a first cDNA strand to be synthesized and an RNA-cDNA heteroduplex to be formed;

d) heat treating the solution containing said RNA-cDNA heteroduplex at a temperature at which said heteroduplex is denatured to form an RNA single strand and a first cDNA single strand without completely inactivating the DNA polymerase activity of said enzyme system;

e) permitting the second primer to hybridize with the first cDNA single strand, followed by synthesis of a second cDNA strand to form a double-stranded cDNA; and

f) denaturing the double-stranded cDNA to form cDNA single strands and subjecting the cDNA single strands to a sufficient number of amplification cycles to obtain a desired amount of amplified product;

wherein after step a), all steps are performed without subsequent addition of any ingredients.

45. (Amended) The method of claim 37, wherein the second primer is permitted to hybridize with the first cDNA single strand at a temperature from about 50° C to about 80° C.

46. (Amended) The method of claim 37, wherein said synthesis of said second cDNA strand occurs at a temperature from about 50° C to about 80° C.

Please add the following new claims 49-53:

49. (New) The method of claim 37, wherein the RNA to be amplified is human immunodeficiency virus RNA.

50. (New) A method for the amplification of RNA in a sample, comprising:

a) obtaining a starting solution by placing, in a container, the sample, a buffer, a first primer, a second primer, a plurality of nucleoside triphosphates, and an enzyme system having reverse transcriptase activity and DNA polymerase activity, wherein the ratio of units of reverse transcriptase activity to units of DNA polymerase activity is 2 to 8;

b) heat treating said solution at a temperature sufficient to permit denaturation of secondary structures that may be present in said RNA but not above 75° C, for a time sufficient to permit denaturation of secondary structures without completely inactivating the reverse transcriptase and DNA polymerase activities of said enzyme system;

c) permitting the first primer to hybridize with the RNA in said solution, followed by synthesis, at a temperature from 45° to 75° C, of a first cDNA strand, thus forming an RNA-cDNA heteroduplex;

d) heat treating the solution containing said RNA-cDNA heteroduplex at a temperature at which said heteroduplex is denatured to form an RNA single strand and a first cDNA single strand without completely inactivating the DNA polymerase activity of said enzyme system;

e) permitting the second primer to hybridize with the first cDNA single strand, followed by synthesis of a second cDNA strand to form a double-stranded cDNA; and

f) denaturing the double-stranded cDNA to form cDNA single strands and subjecting the cDNA single strands to a sufficient number of amplification cycles to obtain a desired amount of amplified product;

wherein after step a), all steps are performed without subsequent addition of any ingredients.

51. (New) The method of claim 50, wherein said enzyme system comprises a first enzyme having reverse transcriptase activity and a second enzyme having DNA polymerase activity.

52. (New) The method of claim 50, wherein the RNA to be amplified is human immunodeficiency virus RNA.

53. (New) The method fo claim 1, wherein the RNA to be amplified is human immunodeficiency virus RNA.